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Pronounced transcriptional regulation of apoptotic and TNF–NF-kappa-B signaling genes during the course of thymoquinone mediated apoptosis in HeLa cells

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Abstract Thymoquinone (TQ) is the active ingredient extracted from the essential oil of *Nigella sativa.* A number of studies implicated TQ as an antitumor agent. In this study, cytotoxic effects of the oil of *N. sativa* and TQ were evaluated on human cervical cancer cell line, HeLa cells. IC_{50} value was ~0.125 μl/ml for *N. sativa* oil preparations and 12.5 μM for TQ. TQ strongly inhibited wound healing at all concentrations ranging from 12.5 to 100 μM in a scratch wound healing assay. Additionally, induction of apoptosis by TQ was assessed by Giemsa staining and TQ was found to induce apoptosis in cancer cells especially at concentrations of 50 and 100 μM. TQ-mediated transcriptional regulation of 84 genes involved in apoptosis was studied using a PCR array. At low dose (12.5 μM), TQ was found to induce expression of four pro-apoptotic genes: BIK (~22.7-fold), FASL (~2.9-fold), BCL2L10 (~2.1-fold), and CASP1 (~2-fold). TQ was also found to reduce the expression of an anti-apoptotic gene implicated in NF-kappa-B signaling and cancer: RELA (~8-fold). At high dose (100 μM), TQ mediated the expression of 21 genes implicated directly in apoptosis (6 genes), TNF signaling (10 genes), and NF-kappa-B signaling (3 genes) such as BIK, BID, TNFRSF10A, TNFRSF10B, TNF, TRAF3, RELA, and RELB. In conclusion, this study implicates the role of TQ in the inhibition of cancer cell proliferation and migration. At the same time, our results strongly suggest that TQ intervenes with TNF and NF-kappa-B signaling during TQ-mediated induction of apoptosis in cancer cells.

Keywords Thymoquinone · Cancer · Apoptosis · TNF signaling · NF-kappa-B signaling

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Introduction

More than 50 % of chemotherapeutic agents used in cancer therapy are natural products or derived from natural products [1]. Thymoquinone (TQ) is the active ingredient extracted from the essential oil of *Nigella sativa* [2]. Numerous studies have been performed to indicate the therapeutic role of TQ in various diseases such as inflammation, cancer, diabetes, and atherosclerosis [3].

A number of studies implicated TQ as an antitumor agent, especially in chemical-induced carcinogenesis in mice [4–6]. TQ was reported to have anti-proliferative effect for many cancer types in cell culture including cancer cell lines of glioma/glioblastoma [7], breast adenocarcinoma [8], leukemia [9, 10], lung cancer [11], colorectal carcinoma [12], pancreatic cancer [13], osteosarcoma [14], and prostate cancer [15].

Thymoquinone triggers apoptosis in colon cancer cells by increasing mRNA and protein levels of P53 and P21WAF1 and by reducing protein levels of BCL-2 [16]. TQ increases the activities of caspases 3, 8, and 9, triggers the release of cytochrome c from mitochondria into the cytoplasm, and increases the protein level ratio of pro-apoptotic Bax to anti-apoptotic BCL-2 in human myeloblastic leukemia HL-60 cells [9]. TQ induces apoptosis in